REMARKS

Claims 14-15, 17, 19-20, 22-25, 27, 29-31, 34-36, 43, 45-47, and 51-55 are pending. Applicants respectfully request reconsideration of the rejections, and allowance of the presently pending claims.

Applicants thank the Examiner for a very helpful interview on May 29, 2003 to discuss the prior art. As discussed in the interview, Applicants have attached herewith, additional copies of the references:

- Ward *et al.* (1995) J. Neuroimmunology **58**:145-155
- Gan *et al.* (2002) J. Neuroscience Methods **121**:151-157
- Der and Lau (1995) P.N.A.S. **92**:8841-8845.

REJECTIONS

Claims 14-15, 17, 19-20, 23-25, 30, 31, 34-36, 43 and 52-53 have been rejected under 35 U.S.C. 103 as being made obvious by Leptin, U.S. Patent no. 6,135,942, in view of Der, U.S. Patent no. 6,077,686.

Claims 19-20 have been rejected under 35 U.S.C. 103 as being made obvious by Leptin, U.S. Patent no. 6,135,942, in view of Der, U.S. Patent no. 6,077,686, and further in view of Petryshyn, U.S. Patent no. 6,124,091.

Claim 22 has been rejected under 35 U.S.C. 103 as being made obvious by Leptin, U.S. Patent no. 6,135,942, in view of Der, U.S. Patent no. 6,077,686, in view of Petryshyn, U.S. Patent no. 6,124,091, and further in view of Kreitman *et al.*, U.S. Patent no. 6,027,876.

Claims 27 and 29 have been rejected under 35 U.S.C. 103 as being made obvious by Leptin, U.S. Patent no. 6,135,942, in view of Der, U.S. Patent no. 6,077,686, and further in view of Villeponteau *et al.*, U.S. Patent no. 6,300,110.

Claims 45-47 and 51 have been rejected under 35 U.S.C. 103 as being made obvious by Leptin, U.S. Patent no. 6,135,942, in view of Der, U.S. Patent no. 6,077,686, and further in view of Staddon *et al.*, U.S. Patent no. 6,312,686.

Claims 54-55 have been rejected under 35 U.S.C. 103 as being made obvious by Leptin, U.S. Patent no. 6,135,942, in view of Der, U.S. Patent no. 6,077,686, and further in view of Panetta *et al.*, U.S. Patent no. 6,251,928.

APPLICANTS' UNEXPECTED RESULTS

Applicants respectfully submit that the present claims are not made obvious by any the cited combination of art. The present claims are drawn to the use of dsRNA comprising at least 100 nucleotides of a candidate gene, for the specific attenuation of expression of genes in mammalian neural cells.

Prior to Applicants' invention, it was known in the art that the introduction of dsRNA of longer than 30 bp into mammalian cells induced an undesirable interferon response. As discussed by Der *et al.* (1995) (attached), double stranded RNA induces interferon expression, which leads to general suppression of gene expression in cell death. Those undesirable side effects meant that long double stranded RNA could not be used to study gene attenuation in mammalian cells. The only dsRNA useful in mammalian cells is short dsRNA, usually about 20-25 bp in length.

It would have been expected that neuronal cells would also show an interferon response with the introduction of long dsRNA, because the prior art publication by Ward *et al.* (1995) J. Neuroimmunology **58**:145-155 (attached), stated "in primary neuronal cultures following exposure of these cells to known IFN-inducing agents, including double-stranded RNA . . . it was found that neurons rapidly express high levels of IFN-β."

Applicants have attached herewith a research article published by co-inventors Li Gan, Kristen Anton and Mirella Gonzalez-Zulueta (Gan *et al.* (2002) J. Neuroscience Methods **121**:151-157), which discusses the unexpected findings of the present invention.

It would have been expected by one of skill in the art that the use of dsRNA of at least 100 nt in length would lead to interferon expression in neuronal cells. And yet, as shown by Applicants, (specification, page 56, last paragraph,ff) specific attenuation was observed in neuronal cells for GFP (green fluorescent protein), and for PARP, both in the levels of the protein produced, and in the functional effects mediated by the protein. Cells transfected with dsPARP-N showed significant protection against OGD-induced cell death compared with mock-transfected cells and those transfected with dsGFP. These results validate the use of RNAi in the analysis of the role of novel and known genes in neurons.

DEFICIENCIES OF THE PRIOR ART

The prior art does not teach or suggest the presently claimed invention. Leptin, U.S. 6,135,942, has been cited against the present claims. The teachings of Leptin are specific to Drosophila (insect) cells. Insect cells do not make interferon. Therefore, the adverse effects of introducing long dsRNA into mammalian cells is not observed with insect cells. Because Drosophila

cells do not share the biology of mammalian cells with respect to induction of interferon by dsRNA, Leptin does not teach or suggest the function of RNAi in mammalian cells.

Der *et al.* has been cited in combination with Leptin. Der *et al.* do not teach the use of double stranded RNA, and therefore cannot teach the presently claimed methods. Der *et al.* teach the use of anti-sense RNA, which is a single stranded RNA, not double stranded. In mammalian cells, the introduction of single stranded RNA does NOT induce an interferon response. Therefore, the effects of double stranded and single stranded RNA are different in mammalian cells.

One of skill in the art is not informed of the present invention by the teachings of Der combined with Leptin.

Petryshyn, U.S. 6,124,091, has been cited in combination with Leptin. Applicants respectfully submit that Petryshyn does not suggest the present invention, alone or in combination with Leptin. Petryshyn teaches an RNA molecule that activates PKR, which is part of the interferon response observed in mammalian cells. Double stranded RNA induces interferon expression, and expression of the double-stranded-RNA dependent kinase PKR mediates interferon activity.

Example 1 of Petryshyn discloses the cDNA synthesis of R-RNA, and does not disclose RNA interference.

Example 11 of Petryshyn describes the use of short anti-sense oligonucleotides, which are single stranded. The RNA used in the present methods are double stranded RNA. As explained above, double stranded and single stranded RNA differ in their ability to induce an interferon response. Because the effects of single stranded RNA are distinct from that of double stranded RNA, one does not predict the activity of the other.

Applicants respectfully submit that Petryshyn does not make obvious the presently claimed invention, and does not remedy the deficiencies of the primary reference, Leptin.

The citation of Kreitman *et al.* is provided for teaching the use of the restriction enzyme RsaI. Applicants respectfully submit that the use of the restriction enzyme is not relied upon for patentability, but is cited as one embodiment of the invention. Kreitman *et al.* does not remedy the deficiencies of the primary reference, which fails to teach or suggest the use of dsRNA of greater than 100 nt in neuronal cells to specifically attenuate gene expression.

Villeponteau *et al.* fail to remedy the deficiencies of the primary reference by failing to teach the use of dsRNA of greater than 100 nt in neuronal cells to specifically attenuate gene expression. The reference teaches the generation of libraries, but not the use of dsRNA in gene attenuation.

Staddon *et al.* does not teach the use of long dsRNA to attenuate gene expression in neuronal cells.

In view of the above amendments and remarks, Applicants respectfully submit that the present invention meets the requirements of 35 U.S.C. 103. Withdrawal of the rejections is requested.

Applicants submit that all of the claims are now in condition for allowance, which action is requested. If the Examiner finds that a Telephone Conference would expedite the prosecution of this application, he is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any other fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815, order number AGYT-013CIP.

Respectfully submitted,

Date: June 9, 2003

By: Pamela Sherwood
Pamela J. Sherwood, Ph.D.
Registration No. 36,677

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile: (650) 327-3231